Hole Density and Capture of Stored-Product Insect Pests in Grain Probe Traps

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ABSTRACT The relationship between number of holes in a grain probe trap body and capture of stored-grain pests was determined in laboratory tests using adults of the rice weevil, *Sitophilus oryzae* (L.), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), and the red flour beetle, *Tribolium castaneum* (Herbst). Polyvinylchloride (PVC) probe bodies were attached to electronic sensor heads, and insect captures were recorded electronically using an Electronic Grain Probe Insect Counter (EGPIC) system. In comparisons among PVC probe trap bodies with 60 – 492 holes, tested at 71 insects per kg in 2.8 kg of soft wheat in cylindrical mini-silos, sawtoothed grain beetle and rice weevil captures were directly related to number of holes in the probe trap body, but there was no relationship for red flour beetle capture. Subsequent tests were conducted comparing sawtoothed grain beetle and rice weevil captures in a PVC probe body with 210 holes over a 40-cm long trapping surface with two commercially available probe traps, a polycarbonate (Lexan) probe trap with 180 holes over a 14-cm long trapping surface and a polyethylene (WBII) probe trap with 750 holes over a 34-cm long trapping surface. The highest percentage capture of both species was in the WBII probe trap, but the 210-hole PVC probe body was as effective as the Lexan probe body for rice weevils and sawtoothed grain beetles at 71 and 17 insects per kg of wheat, respectively.

KEY WORDS *Tribolium castaneum, Oryzaephilus surinamensis, Sitophilus oryzae,* automated monitoring, grain probe trap

GRAIN PROBE TRAPS were first developed by Loschiavo and Atkinson (1967) for detection of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), in stored grain in Canada. Before development of probe traps, insect presence in stored grain was determined by removing representative grain samples using a grain trier and then manually sieving the insects from the grain. Low-level insect infestations were often missed unless a heavily-infested pocket was sampled. However, the removal of grain disrupted insect populations, which led to errors in understanding insect distribution in the grain mass.

The original probe trap was described as "a rocket-shaped, escape-proof cylinder that lets insects in but keeps grain out" (Chemelli 1974). The trap was machined from solid brass and included a hollow cylinder made from a 14-gauge brass sheet. A series of 2-mm diam holes, spaced 1.6 mm apart, were drilled in the brass sheet with holes in adjacent columns offset vertically. The cylinder with holes was ≈4.1 cm diam × 7.7 cm long. A modified version of the trap was made with more inexpensive materials, and the section of the cylinder with holes was slightly shorter and narrower than the original (Chemelli 1974). This trap did not withstand the pressure of the grain, so a third version of the trap was made, which was slightly longer and much narrower (Loschiavo and Atkinson 1973). The cylinder with holes was 2.5 cm diam × ≈8.3-cm long, and the holes were increased in size to 2.16 mm diam. Unlike the first version of the probe trap, the second and third versions had holes lined up horizontally and vertically so that they formed a grid of holes (Chemelli 1974).

Barak and Harein (1982) modified the basic design by increasing the hole size to 2.39 mm to allow capture of the larger black flour beetle, *Cynaeus angustus* (LeConte), as well as other stored-grain beetles. The same number of holes were drilled in a column as in the trap developed by Loschiavo and Atkinson (1967), but because of the larger hole size, the length of the trapping surface was increased. Wright and Mills (1984) evaluated an increase in hole size from 2.8 to 3.8 mm diam and found that the larger holes improved insect capture, but further increasing size to 4.8 mm diam had no effect. Burkholder (1984) developed the first commercial perforated probe trap (Grain Probe

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Insect Trap, ThermoTrilogy, Columbia, MD). It was made from a 2.5 cm diam × ≈14-cm long cylinder of clear polycarbonate (Lexan) plastic with 2.79-mm diam holes. The Lexan cylinder had 3.2-mm thick walls, which allowed the holes to be drilled at a slope of 50° downward angle. Like the original Loschiavo and Atkinson (1967) probe trap, the adjacent columns of holes were offset vertically. The length of the entire cylinder was 38 cm, and the bottom half of the cylinder, which had no perforations, contained a plastic funnel placed over a plastic graduated test tube that retained trapped insects. Subsequently, a second commercially available probe trap was made from a perforated section of tubular polyethylene (Barak et al. 1990; Storgard WBII probe trap, Trécé, Salinas, CA). It is the largest of the perforated probe traps, with a grid of 4 mm × 3 mm rectangular openings on a 3.0 cm diam × ≈34-cm long cylinder. White et al. (1990) provide a review of trap development and an overview on research on the use of probe traps in stored grain.

Although grain probe traps have advantages over bulk grain sampling, there are problems associated with use of these devices. Traps must be removed from the grain bin and inspected periodically to determine the number of insects that have been captured. This is labor intensive, limits the temporal availability of data, and restricts placement of the probe traps to easily accessible locations.

The Electronic Grain Probe Insect Counter (EGPIC; Shuman et al. 1996, Litzkow et al. 1997) was developed to overcome these limitations. EGPIC uses grain probe traps that are modified by the addition of infrared-beam sensor heads attached to the bottom of the probe trap bodies. An electronic count is generated whenever an insect that has crawled into a probe trap falls through the sensor head, and this information is transmitted to a computer via beam generation/detection circuitry. Prototype versions of EGPIC first used the WBII probe trap (Shuman et al. 1996) and then the clear Lexan probe trap (Arbogast et al. 2000) for the probe trap body. The Lexan probe trap was used in the inverted position to reduce entry of grain particles (Subramanyam et al. 1989), which would generate additional electronic counts. These initial studies used sensor probes that were custom-made in-house at the ARS laboratory in Gainesville, FL. Subsequent research used sensor heads that were produced by small-scale manufacturing processes combined with Lexan probe bodies as a step toward development of a commercially available EGPIC system (Epsky and Shuman 2000).

For the commercially produced EGPIC sensor probes, the sensor head and the probe trap body will be manufactured as a single multipiece injection-molded unit, which will be more cost effective and will allow production of an alternative to the WBII probe trap and the Lexan probe body configurations. The Lexan probe trap is a cylinder with a 14-cm long trapping surface that has 180 evenly spaced holes. Availability of a probe body with a greater length of trapping surface, something equal to or greater than the longer WBII probe trap, may increase the effectiveness of the electronic probe trap and provide better coverage in the grain. It would also allow for an increase in number of entry holes into the grain probe trap. In comparisons of the WBII probe trap and the Lexan probe trap, the WBII probe traps tend to capture more insects, but the differences were not always significant (Barak et al. 1990; Subramanyam et al. 1993; Fargo et al. 1994). In addition to differences in hole size, shape and angle, there are also differences in hole spacing (i.e., offset versus adjacent holes) between the two probe traps. Little is known about the effects of these other factors. Therefore, research was conducted to determine the relationships between hole number and spacing and target insect capture and to compare capture among WBII probe traps, the EGPIC sensor heads with Lexan probe trap bodies, and the EGPIC sensor heads with manufactured probe trap bodies.

Materials and Methods

Insects used in this study included 2–4-wk old adults of the rice weevil, *Sitophilus oryzae* (L.), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), and the red flour beetle, *Tribolium castaneum* (Herbst). Most insects were from laboratory colonies that have been maintained at the USDA/ARS laboratory in Gainesville, FL, for at least 20 yr. Additional tests were conducted with a field strain of red flour beetles that was started from beetles field collected 3 yr before the tests. Wheat used in this study was organic soft wheat,and separate supplies of wheat were used for each species. After a trial, the wheat was sieved (10-mesh screen) to remove all insects and dockage. Grain was kept in a freezer between tests to kill all eggs or developing larvae and to preserve the grain. Grain removed from the freezer was held at room temperature for 24 h before use.

Test probe bodies were produced from dark gray polyvinylchloride (PVC) cylinders that were the same diameter (2.5 cm) as the Lexan probe traps. The test probe bodies were fabricated using a computer-controlled step and repeat drilling apparatus custom designed by Analytical Research Systems (Gainesville, FL). The 2.79-mm diam holes were precision-drilled at an upward 45° angle on the 40-cm-long trapping surface. One set of precision-drilled probe bodies had six columns that ran the length of the trapping surface and 10, 21, and 41 holes per column for a total of 60, 126, and 246 holes, respectively. A second set of precision-drilled probe bodies had 12 columns and 5, 11, 21, and 41 holes per column for a total of 60, 132, 252, and 492 holes, respectively (Fig. 1). This resulted in probe bodies in which the holes were adjacent (6 columns) or offset (12 columns). The holes were evenly spaced along the trapping surface. These precision-drilled probe bodies were threaded to electronic sensor heads, and insect counts were recorded electronically.

Tests comparing insect capture among different probe bodies were conducted in mini-silos made from PVC cylinders (10 cm diam × 50 cm tall). A single grain probe trap was placed in the center of the mini-

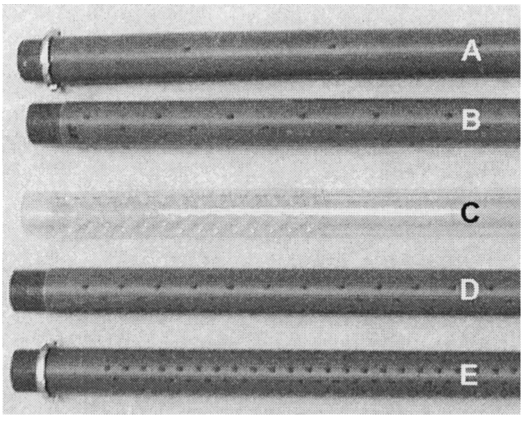

Fig. 1. Precision-drilled polyvinylchloride (PVC) probe bodies used with electronic sensors. Shown here are the 12-column configurations with 60, 132, 252, and 492 holes (A, B, D, E, respectively) and the Lexan probe body (C). The threaded section at the bottom of the probe body was used to attach the probe body to an electronic sensor head for use with the electronic grain probe insect counter (EGPIC) system.

silo, with the bottom of the trap extending through a hole in the bottom of the mini-silo. A sleeve made from clear acetate sheeting was placed around the probe body to prevent insects from entering the probe traps before the electronic counts were initiated. The trap receptacle was attached to the probe trap on the outside of the mini-silo, so that it could be checked without disturbing the grain in the mini-silo. The trap receptacle was coated with Teflon (polytetrafluoroethylene) to prevent captured insects from moving back into the sensor probe. Wheat and insects were placed in the mini-silos in a half-and-half layering protocol in which half of the grain, half of the insects, the second half of the grain, and the second half of the insects were added. For most experiments, sleeves were lifted immediately, and electronic counting was initiated. However, a 24-h lag was added before the sleeves were lifted in experiment 2 for comparative purposes. Number of insects captured was determined by emptying the trap receptacle after 24, 48, and 72 h. Electronic insect counts were used to compare periodicity of insect capture among the different PVC test probes and among the different species.

Experiment 1 compared capture of red flour beetles, sawtoothed grain beetles, and rice weevils in EGPIC sensor heads fitted with the seven PVC test probe bodies. One species was tested at a time at a density of ≈71 insects per kg of wheat. The mini-silos were filled with grain (2.8 kg) to a level slightly above the top row of holes. There were five replicates of tests with red flour beetles, and three replicates each of tests with sawtoothed grain beetles and rice weevils. Experiment 2 was conducted to further evaluate capture of red flour beetles. For this test, two sets of probe bodies with 60, 132, 252, and 492 holes were tested with the sleeves lifted immediately after the addition of the insects and grain or with sleeves lifted 24 h later, providing a lag period for insects to distribute in the grain before the initiation of the test. Separate tests were conducted with the laboratory strain and the field strain of red flour beetles, and there were two replicates for each strain.

Based on the results of experiment 1 and cost factors, it was decided that the precision-drilled probe bodies for the commercial probes would have 10 columns of 21 holes per column, for a total of 210 holes over a 40-cm-long trapping surface. The number of columns was reduced from 12 to 10 to provide room for two vertical groves to run the cables connecting the electronic sensors in the sensor head to the circuit board in the top of the body. Tests were conducted to compare capture in the EGPIC sensor heads attached to 210-hole probe bodies—the EGPIC sensor heads attached to the Lexan probe body and the WBII probe traps. Using one species at a time, tests were conducted with sawtoothed grain beetles and rice weevils. Influence of trapping surface differences was also evaluated by testing the smaller Lexan probe trap body in 1.9 kg of wheat, which filled the mini-silo to just above the top row of holes, and in 2.8 kg of wheat, the same amount of grain as the mini-silo with the precision-drilled probe trap body. When used with the greater amount of grain, the top row of holes on the Lexan probe trap body was ≈13 cm below the surface of the grain. The WBII probe traps were tested in 2.3 kg of wheat, which filled the mini-silo to just above the top row of holes. The protocol was the same as that used in experiment 1. There were four treatments, and the test was replicated six times. Experiment 4 was the same as above, but only sawtoothed grain beetles were tested, and insect density was reduced to ≈17 beetles per kilogram of wheat. This test was also replicated six times.

Number of insects captured within the three consecutive 24 h time periods and total number captured over the 72-h time period were converted to percentage captured to standardize the data before analysis. Data from experiment one were analyzed with a homogeneity-of-slopes model using Proc GLM (SAS Institute 1985) with separate analyses conducted for each species and each time period tested for the tests. Number of columns was tested as an analysis of variance (ANOVA) factor and number of holes was tested as a regression model. Effects of single factors were evaluated with two-sample *t*-test analysis using Proc *t*-test or with linear regression using Proc REG (SAS Institute 1985). Data from experiment 2 were analyzed with factorial analysis of variation (ANOVA) with strain type (2 levels), lag (2 levels), and number of holes (4 levels) as main factors and all two-way interactions using Proc GLM. Data from experiments 3 and 4 were analyzed with separate one-way ANOVAs for each species and/or insect density. Data were subjected to the Box-Cox procedure, which is a power transformation that regresses log-transformed standard deviations (y) against log-transformed means (x) (Box et al. 1978), and data were transformed to stabilize the variance before analysis when necessary. Information from time-stamped electronic insect

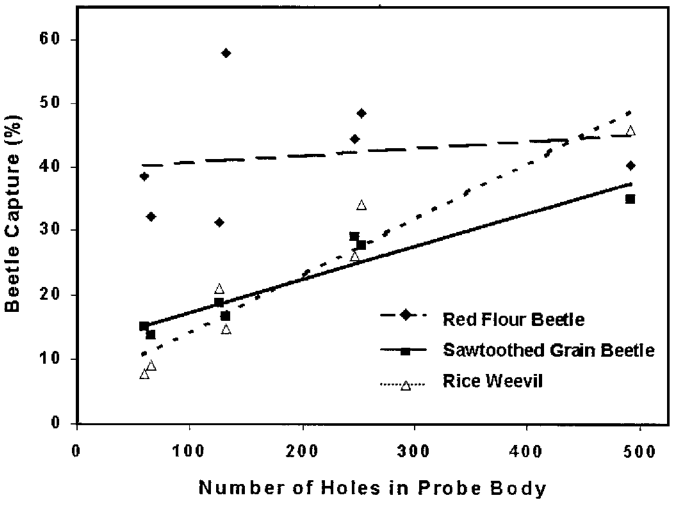

Fig. 2. Percentage of red flour beetles, sawtoothed grain beetles, and rice weevils captured in 72 h in probe traps made from precision-drilled PVC cylinders with increasing number of holes over a 2. 5 cm diam × 40 cm long trapping surface. Data points are the average capture of each species in five (red flour beetles) and three (sawtoothed grain beetles and rice weevils) replicate trials. All tests began with 200 adult beetles in 2. 8 kg of wheat, and tests were conducted in a laboratory.

counts recorded by the EGPIC system was evaluated by partitioning capture into consecutive 6-h time periods and determining capture frequency over the 72 h of a test. Chi-square analysis using Proc Freq of a 3 × 24 contingency table of capture by species and by time period was used to compare capture of the three species.

Results

Percentage capture of red flour beetles over 72 h was not affected by the number of holes in the probe body (Fig. 2). There were higher percentages of red flour beetles captured in probes with 12 columns than in probes with 6 columns during the first 24-h sampling period (30.3 ±19.55 versus 17.1 ± 8.21; $t = 2.4245$, df = 21.3, $P = 0.0243$) and over the entire 72-h sampling period (49.0 ± 24.26 versus 34.1 ± 14.37; $t = 2.2543$, df = 31.6; $P = 0.0313$) but no differences in the second and third 24-h sampling periods. A direct relationship between number of holes and percentage captured over 72 h was observed for sawtoothed grain beetles and rice weevils (Fig. 2), and this was true for all earlier sampling periods. Number of columns had no effect on capture of either species. The regression model for percentage capture (y) over a 72-h sampling period versus number of holes (x) in the probe trap body was $y = 5.28 + 0.088x$ ($r^2 = 0.657$, $P = 0.001$) for sawtoothed grain beetles, and $y = 12.08 + 0.052x$ ($r^2 = 0.601$, $P = 0.001$) for rice weevils.

Periodicity of insect capture was determined by using the time stamp data that were recorded by the EGPIC system. Total captures were partitioned into number captured for each 6-h time period beginning at 1 p.m. on day 1 (± 1 h depending on the individual trial) and ending at noon on day 3. In some of the tests with red flour beetles, there were high numbers of over-counts, that is, more electronic counts than number of insects recorded. Because over-counts tend to be associated with high capture rates and do not accurately reflect amount of insect activity, all electronic counts that were 10% higher than the actual insect count were deleted from subsequent evaluation of time stamp data. As shown in Fig. 3, red flour beetle capture was highest during the first 6 h of the test, with >30% of total capture occurring during this time period. Percentage capture rapidly dropped off, but slight increases in capture occurred during the day on both day 2 and day 3. Sawtoothed grain beetle capture was also highest early in the test, with a broader time period of peak capture and a high percentage capture occurring over the first 18 h of the test. Capture was the lowest during the day, with an increase in capture during the second night and a small increase during the third night. Rice weevils showed the lowest initial capture but the strongest periodicity. Peak capture was obtained between 1 a.m. and 7 a.m. on all three nights, with the highest capture occurring on the last night of the test. These differences in periodicity among the three species were significant ($\chi^2 = 558.55$, df = 22, $P = 0.001$). The primary differences were observed with higher-than-expected capture of red flour beetles and lower-than-expected capture of rice weevils during the first 6 h, and higher-than-expected capture of rice weevils during the last 12 h of the test.

In experiment 2, the comparison among the probes with 12 columns, no effect of number of holes was observed for the field strain red flour beetles or in trials that had a 24-h lag before initiation of the test. There was an interaction between the effect of strain and lag time on percentage captured during the first 24 h of the test, so the factors of strain and lag time were combined, and separate one-way ANOVAs were conducted within each time period (Table 1). The great-

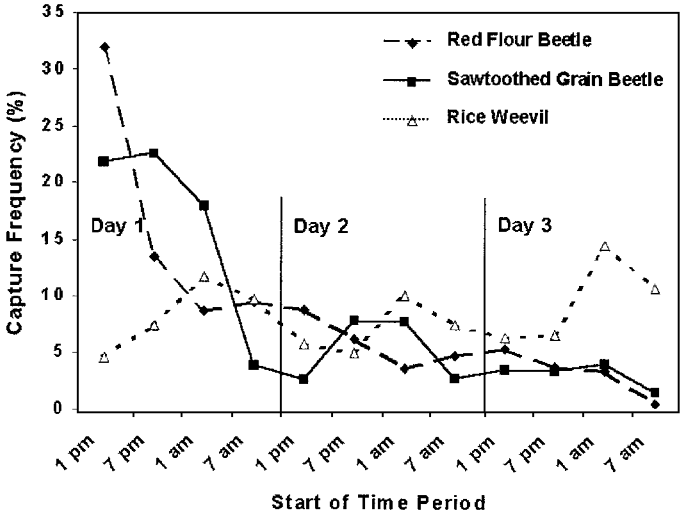

Fig. 3. Periodicity of capture of red flour beetles, sawtoothed grain beetles, and rice weevils captured in probe traps made from precision-drilled PVC cylinders attached to electronic sensor head which recorded time-stamp data on capture. All tests were started at 1 p.m. ±1 h and collections were continued for 3 d. A total of 1276 red flour beetles, 912 sawtoothed grain beetles, and 676 rice weevils were captured in these tests.

est capture occurred during the first 24 h of the test, and the highest capture was in tests of field strain beetles tested without a lag period. Laboratory strain beetles with or without a lag period had the lowest capture, and field strain beetles tested with a lag period were intermediate in capture for the first 48 h of the test.

Experiment 3 compared capture in the PVC probe trap with 210 holes with capture in commercially available probe traps. Treatment affected percentage capture of rice weevils and sawtoothed grain beetles (Table 2). Rice weevil capture in the PVC probe trap was equal to capture in the Lexan probe trap when tested in the smaller amount of grain, and intermediate to capture in the other two probe trap/amount of grain treatments. However, capture of sawtoothed grain beetles was lowest in the PVC probe trap. There tends to be lower capture of rice weevils than sawtoothed grain beetles in probe traps (e.g., Shuman et al. 1996), and it was hypothesized this contributed to differences in the tests of the two species. Therefore, experiment 4 evaluated capture of a lower density of sawtoothed grain beetles. Again, treatment affected percentage capture (Table 2). When sawtoothed grain beetles were tested at a lower density, there were no differences among the PVC and Lexan probe traps, which all captured a lower percentage of insects than the WBII probe traps.

Discussion

Although several combinations of size and number of holes have been used in grain probe traps, there have been few studies that evaluated effects of changes in these factors on number of insects captured. Some studies have found no difference with number of holes, and others have found greater capture in probe traps with more holes. Toews and Phillips (2002) found that increase in number of holes (from 40 to 120 holes along a 15-cm-long probe body) did not affect capture of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), and an equal capture of this species in EGPIC sensor probes with the Lexan probe bodies as in WBII probes (Toews et al. 2003). In our studies, the relationship between hole density and insect capture was affected by insect species tested and by the density of the population. No relationship between number of holes and capture of red

Table 1. Mean (± standard error) percentage of red flour beetles captured in precision-drilled polyvinylchloride probe traps in consecutive 24-h time periods within a 72-h laboratory test

Time period, h	Laboratory strain beetles		Field strain beetles		F	P
	No lag	24-h lag	No lag	24-h lag		
0–24	21.4a ± 2.98	27.9a ± 5.13	48.4b ± 6.63	33.8a ± 4.05	5.56	0.0040
24–48	7.6ab ± 1.67	6.75a ± 0.77	12.3c ± 1.28	10.6bc ± 1.10	4.23	0.0138
48–72	7.3a ± 0.80	5.4a ± 0.98	7.0a ± 1.20	6.8a ± 0.88	0.74	0.5380

Insect density was 71 insects per kg, and tests were conducted in mini-silos containing 2.8 kg of wheat. Tests were initiated immediately after insects and grain were added to the test arena (no lag) or after 24 h to allow insects to distribute in the grain.

Means followed by the same letter within a row are not significantly different (LSD mean separation test, $P = 0.05$; df = 3, 28).

Table 2. Mean (± standard error) percentage of beetles captured in a 210-hole precision-drilled polyvinylchloride (PVC), a polycarbonate (Lexan; Grain Probe Insect Trap) and a polyethylene (Storgard WBII) probe trap in 72-h laboratory tests, amount of wheat used is given in parentheses below trap type

Species	Insect density, per kg	PVC probe body (2.8 kg)	Lexan probe body (2.8 kg)	Lexan probe body (1.9 kg)	WBII probe body (2.3 kg)	*F*	*P*
Rice weevil	71	28.8b ± 1.70	20.5a ± 0.92	33.2b ± 3.42	61.8c ± 4.16	42.02	0.0001
Sawtoothed grain beetle	71	21.8a ± 2.12	48.5b ± 5.25	40.3b ± 4.50	90.7c ± 1.69	50.09	0.0001
Sawtoothed grain beetle	17	38.7a ± 7.69	49.7a ± 4.42	49.5a ± 4.20	95.5b ± 2.22	8.40	0.0008

Means followed by the same letter within a row are not significantly different (LSD mean separation test, $P = 0.05$; data were square-root transformed [tests of 71 insects per kg] or log-transformed [test of 17 insects per kg] before analysis; untransformed means and standard errors are shown, df = 3, 20).

flour beetles was observed. Red flour beetles prefer broken grains and flour, so intact wheat, especially intact wheat that has been sieved frequently, represents an unfavorable environment. Thus, red flour beetles moved rapidly out of the grain and may have been less influenced by the number of holes. This rapid movement was observed in the time-stamped data as most of the red flour beetle capture occurred within the first 24 h of the trial. The laboratory strain red flour beetles used in this study tend, as adults, to stay on the surface of the rearing medium, whereas the field strain adults tend to move down into the diet. This difference in behavior may have contributed to the higher capture of the field strain red flour beetles obtained in these studies.

A direct relationship between number of holes and number of insects captured was obtained for sawtoothed grain beetles and rice weevils. Sawtoothed grain beetles have similar feeding habits as red flour beetles in that they need cracked or damaged grain and fine material for optimal development (Fleming 1988). Peak capture occurred early in the test period, although this capture was spread out over the first 18 h as opposed to the first 6 h for red flour beetles. The slower capture of sawtoothed grain beetles may explain the direct relationship between total capture and number of holes for sawtoothed grain beetles but not for red flour beetles. In contrast, rice weevils are internal feeders, and intact grain is a suitable environment for rice weevils. The rice weevils were the slowest to be captured in the probe traps, and rate of capture was increasing toward the end of the test period.

Capture in grain probe traps is dependent on insect activity, and factors such as insect density, trap depth, temperature, trapping duration, and so forth, all affect number of insects captured (White and Loschiavo 1986, Fargo et al. 1989). Thus, comparisons among different probe trap types will be affected by the conditions of the test. This was observed in the test of the 210-hole PVC probe body in comparison with the WBII and the Lexan probe traps. Although the WBII traps captured the highest percentage of insects in all tests, the 210-hole PVC probe body was as effective as the Lexan probe body for rice weevils and sawtoothed grain beetles at 71 and 17 insects per kg of wheat, respectively. Field tests will be needed to fully evaluate the effectiveness of the 210-hole PVC probe body versus other probe traps.

Grain probe traps are primarily used to detect insect infestation in stored grain (Barak and Harein 1982), with grain probe samples (deep bin cup or grain trier) used to estimate insect population density (Hagstrum et al. 1985). Additional research has related capture in grain probe traps to insect density (Lippert and Hagstrum 1987, Subramanyam and Harein 1990, Subramanyam and Hagstrum 1995) as steps toward developing predictive systems. Interpretation of probe trap data has remained difficult, primarily because of the dynamic nature of the stored-grain environment. Availability of an electronic grain probe as part of an EGPIC system, which is combined with automated temperature and/or moisture monitoring systems, will provide additional information that can be used to improve trap interpretation. Transmission of count data to an off-site computer will allow immediate notification of insect activity to the grain manager without having to enter the grain bin and will allow insect monitoring during fumigation or other pest control activities. Availability of time-stamp data will allow evaluation of insect activity increase or decrease as well as determination of periodicity of activity that, when considered with accompanying temperature/moisture data, may improve trap interpretation and lead to improved management decisions.

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